

Bacterial Growth Experiment

Pseudomonas fluorescens, the bacteria used in the laboratory exercise you will begin soon, does not cause disease in healthy people. However, people who have weakened immune systems should not have contact with most microorganisms or with people who handle those organisms. Your immune system may be weakened if you are undergoing antibiotic therapy, if you are taking immunosuppressive drugs or drugs for cancer treatment, or if you have AIDS or are HIV-positive. If you have a weakened immune system for these or any other reasons, let your teacher know and he or she will provide you with an alternative experience that is safer for you.

Follow the directions below to test the hypothesis using the bacterial species *Pseudomonas fluorescens* and the antibiotic kanamycin. The flow chart on the last page provides an overview of the experiment.

Hypothesis:

DAY 1

1. Collect the following materials from your teacher:

- 1 test tube culture of *P. fluorescens* (the parental culture)
- 1 test tube containing nutrient broth
- 1 test tube containing nutrient broth with kanamycin
- 1 nutrient agar plate
- 1 nutrient agar plate with kanamycin

You will need the following materials at your laboratory station: 4 sterile 1-milliliter pipets, pipet pump or bulb, container with disinfectant for disposing of used pipets, Bunsen burner, grease pencil for labeling, and beaker of alcohol with a bent glass rod spreader.

2. For your safety and the success of your experiment, you must use aseptic techniques when handling bacterial cultures. You must also discard used cultures safely. Your teacher will explain and demonstrate aseptic techniques and indicate where you should discard your used cultures (with caps and lids in place). Your teacher will decontaminate all of the cultures before disposal.

Swirl the *P. fluorescens* culture gently to distribute the bacterial cells evenly. Then follow your teacher's instructions for maintaining sterile conditions while transferring 0.1 milliliter from the culture into the test tube of nutrient broth and into the test tube of nutrient broth with kanamycin. Label the first test tube "A" and the second test tube "B."

3. Swirl the *P. fluorescens* culture again and follow your teacher's instructions to deposit 0.1 milliliter from the culture on each of the nutrient agar plates. Use a sterile, bent glass rod to spread the culture evenly over the surface of the plates. Label the nutrient agar plate "1" and the nutrient agar plate with kanamycin "2."
4. After the culture has soaked into the plates (about 5 to 10 minutes), invert the plates and incubate them and the two broth cultures at 25°C for three days.

DAY 2 (3 days later)

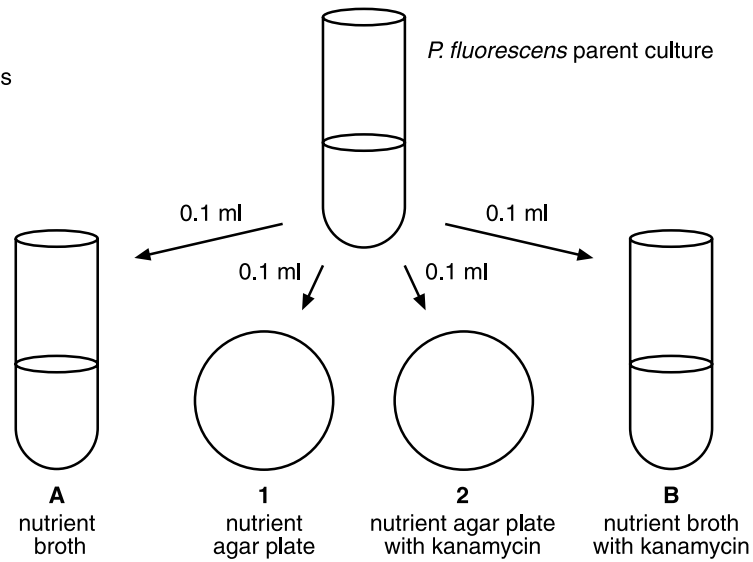
5. Retrieve the broth cultures (A and B) from the first session and collect 2 new nutrient agar plates and 2 nutrient agar plates with kanamycin. Check that you have 4 sterile 1-milliliter plates, pipet pump or bulb, pipet disposal container, Bunsen burner, and alcohol with a bent glass rod spreader.
6. Swirl culture A gently and follow the procedure in Step 3 to prepare two plates, one nutrient agar plate and one nutrient agar plate with kanamycin. Label the first plate "3" and the second plate "4."
7. Swirl culture B gently and repeat Step 6 using samples from this culture. Label the nutrient agar plate "5" and the nutrient agar plate with kanamycin "6."
8. After the culture has soaked into the plates, invert them and incubate them at 25°C for two or three days. Dispose of the A and B cultures as your teacher directs.

DAY 3 (2-3 days later)

9. Collect all six plates and draw the amount of bacterial growth on each plate on the flow chart.

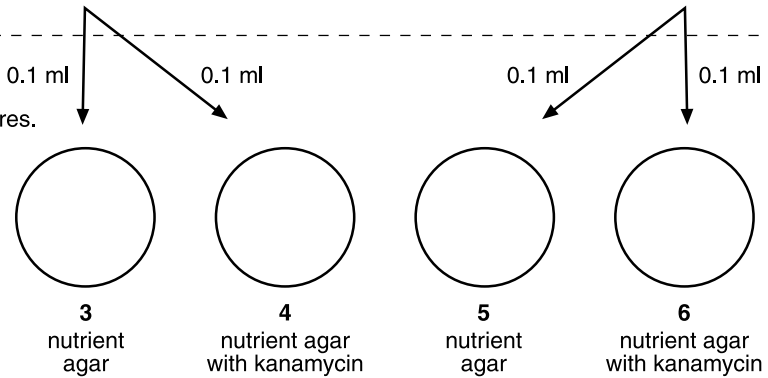
Day 1

Steps 1 to 4:
Prepare 2 broth cultures
and 2 plate cultures.



Day 2

Steps 5 to 8:
Prepare 4 plate cultures.



Day 3

Step 9: Collect plates and record results above.

Discussion Questions for the Bacterial Growth Experiment

Refer to the results from your bacterial growth experiment as you answer the following questions.

1. Compare the bacterial growth on the two plates from the parental culture (Plates 1 and 2). Which has more growth? Explain why. How do you explain the presence of bacteria on the plate containing kanamycin?
2. Compare the growth on Plates 3 and 4, which you prepared from culture A (without kanamycin). How does the growth on the plates with and without kanamycin appear? What does this tell you about the bacteria grown in culture A?
3. Compare the growth on Plates 5 and 6, which you prepared from culture B (with kanamycin). How does the growth on the plates with and without kanamycin appear? What does this tell you about the bacteria grown in culture B?
4. Compare the growth of cultures A and B on Plates 4 and 6 (with kanamycin). Explain how culture B could have so many more resistant bacteria than culture A, even though they both came from the same parental culture.
5. How do you explain the presence of some resistant bacteria in the parental culture and culture A?

Debi's Story: Explaining What Happened

Follow the steps below to explain what happened to Debi French.

1. Click on *The Diagnosis* and view Debi's description of her initial diagnosis. Summarize what you learned by completing the following sentences:

Debi contracted tuberculosis (TB) from

The symptoms Debi had were

2. Click on *The Initial Treatment* to hear Debi describe the treatment prescribed by her doctor and its outcome. Summarize what you learned by completing the following sentences:

The treatment to cure TB is

When Debi started the treatment

3. Review the results and conclusions you drew from Plates 1–4 of your bacterial growth experiment. Put together those conclusions with the observations from the first two parts of *Debi's Story* and complete the following sentence:

Debi's health began improving when she started the drug therapy for TB because

4. Click on *The Treatment Fails* to learn what happened to Debi next. Summarize what you learned by completing the following sentences:

On Valentine's Day 1994, Debi learned

The drugs Debi took to cure her TB were not working because

5. Review the results and conclusions from Plates 5 and 6 of your bacterial growth experiment. Put together those results and Debi's experience to complete the following sentence:

Debi had a relapse (developed an active case of TB again), even though her health had improved and she was still taking the drugs to cure TB, because

6. Click on *A Happy Ending* to learn what finally happened to Debi. Summarize what you learned by completing the following sentences:

Debi was finally cured of TB by

Debi's warning about infectious diseases like TB is

Antibiotic Concerns

Each of these statements describes a potentially inappropriate use of antibiotics. How would you persuade people to eliminate unnecessary use of antibiotics in these cases?

Statement 1

In response to pressure from patients to “give me something,” some doctors prescribe antibiotics before they know whether a patient’s illness is caused by a virus or a bacteria.

Statement 2

Antibiotics are widely used in livestock feed to improve the growth of animals.

Statement 3

A popular marketing strategy for some products intended for healthy people (for example, hand soaps and children’s toys) is to include antibacterial drugs in the products.